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Study on mutual interactions and electronic structures of hyaluronan with Lysine, 6-Aminocaproic acid and Arginine

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ABSTRACT

Interactions between polyelectrolytes and oppositely charged surfactants have been in a great interest for several decades, yet the conventional surfactants may cause a problem in medical applications. Interactivity between polysaccharide hyaluronan (HA) and amino acids Lysine, 6-Aminocaproic acid (6-AcA), and Arginine as an alternative system is reported. The interactions were investigated by means of rheology and electric conductance and the electronic structures were explored by the density functional theory (DFT). Lysine exhibits the strongest interaction of all, which was manifested, e.g. by nearly 6-time drop of the initial viscosity comparing with only 1.3-time lower value in the case of 6-AcA. Arginine interaction with HA was surprisingly weaker in terms of viscosity than that of Lysine due to a lower and delocalized charge density on its guanidine group. According to the DFT calculations, the binding of Lysine to HA was found to be more flexible, while Arginine creates more rigid structure with HA.

1. Introduction

The mutual physical interaction between polysaccharide hyaluronan and amphiphilic molecules such as more or less conventional surfactants has been in a great interest for nearly three decades. The reason is unique properties and potential applications of this linear and naturally occurred polysaccharide that is composed of repeating disaccharide units made of D-glucuronic acid (or its salt form) and D-N-acetylglucosamine linked by β-(1–3) glycosidic bonds. The disaccharide units are alternatively linked by β-(1–4) glycosidic bonds (Lapčík, Lapčík, De Smedt, Demeester, & Chabreček, 1998; Nieduszynski, 1985; Prehm, 2002). The potential applications, which have already been used in a certain measure, are drug targeting delivery (Jaracz, Chen, Kuznetsova, & Ojima, 2005; Vercruysse & Prestwich, 1998) using hyaluronan as a carrier and a transport agent due to its specific binding to some cell receptors, namely CD44. However, most of these examples of applications were based on a chemical modification of the polysaccharide (Mlčochová et al., 2006, 2007; Šoltés et al., 1999) in order to shift its behavior more toward an amphiphilic polymer able to cluster its additional hydrophobic side chains to form polymeric micelles. Such micelles are assumed to interact with bioactive molecules, mostly hydrophobic, e.g. drugs, antibiotics, etc., in terms of their encapsulation into the micelles hydrophobic interior.

A chemical modification can considerably change some properties of the original polymer, such as its water solubility or biodegradability. Other alternative is a physical "modification" by means of a physical interaction or a binding of hyaluronan with micelle forming species, i.e. surfactants. The group of prof. Lindman and others have focused on the study of phase behavior, interactions and encapsulation capabilities of hyaluronan and cationic surfactants, based on N-alkytrimethylammonium salts (Thalberg & Lindman, 1989; Thalberg, Lindman, & Karlström, 1990; Santos, Nome, & Zanette, 1994). The authors well described the phase behavior and constructed phase diagrams having found that the phase separation occurs very soon in pure water, while in the presence of low molecular salt the separation is shifted to larger surfactant concentrations. The binding itself is in the form of micelle-like clusters, as has also been observed for a large number of polymer-surfactant systems. The value of pH also strongly influences the phase behavior, particularly when being close to the pK of the HA carboxylic groups, i.e. at around 2.5, at which the interaction is practically screened out (Chytil, Strand, Christensen, & Pekař, 2010). Furthermore, a decrease in the relative viscosity was observed (Herslöf, Sundelöf, & Edsman, 1992) and a decline from the linear shape of the electric conductance dependence on the

Abbreviations: AA, amino acid(s); 6-AcA, 6-Aminocaproic acid; CMC, critical micelle concentration; DFT, density functional theory; HA, hyaluronan (hyaluronic acid); PBS, phosphate buffer solution; RHAMM, receptor for HA-mediated mobility.

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surfactant concentration (Thalberg & Lindman, 1989) as evidence of the mutual interaction.

Later studies report about the interactions of HA with dimeric cationic surfactants, e.g. alkanediyl-α,ω-bis(dimethylammonium bromide), showing different effects in comparison to monomeric surfactants; e.g. slight shear-thickening rheological behavior or initial increase in relative viscosity at low surfactant concentrations (Pisárčik, Soldán, Bakoš, Devínsky, & Lacko, 1999), or unusual parabolic dependence of the surface tension on log surfactant concentration in the presence of HA (Pisárčik, Tokoyoko, Devínsky, Lacko, & Bakoš, 2000).

The conventional surfactants may cause a problem due to their harmful effect on cells and their toxicity in general, thus amino acids or amino-acid-based surfactants may be appropriate alternative. In contrast to a large number of publications on HA–surfactants interactions, little has been published about the interactivity of HA with free amino acids or amino groups-containing molecules over the last 25 years. Nevertheless, there is a great deal the literature focused on HA-binding proteins and peptides such as cell receptor CD44 (Favreau, Faller, & Guvench, 2013) and RHAMM (receptor for HA-mediated mobility; Yang, Zhang, & Turley, 1993), cartilage proteoglycan aggrecan (Ching Hen, Gribbon, Day, & Hardingham, 2008), or Link module from TSG-6 (Blundell et al., 2003; Kahman et al., 2000) associated with inflammation. The interactions between HA and proteins are most commonly mediated by a domain of approximately 100 amino acids, which is termed as a Link module; however, not all HA-binding proteins (e.g. RHAMM) contain this domain. The data also supports the importance of ionic interactions between the two molecules and that the charge spacing in the Link module or amino acid cluster is important for recognition of HA.

The study of the interactivity between HA and alkylated amino acids, i.e. serine dodecylester, glycine dodecylester, glutamate didecylester, and aspartate dioctylester, by means of fluorescence displayed a similar aggregation behavior of these amino acids as conventional surfactants (Halasová, Mravec, & Pekař, 2013). In this study the CMC of the alkylated amino acids was lowered by one order within the magnitude in the presence of HA or in 0.15 M NaCl. On the other hand, no such effect was observed in the case of the aspartate derivative and no aggregation behavior of the glutamate derivative was detected whatsoever, due to its low solubility.

This paper reports about the interactivity between HA and amino acids Lysine, 6-Aminocaproic acid (6-AcA), and Arginine in aqueous solutions by means of rheology, electric conductance measurements, and molecular dynamic simulations/calculations. The aims is to detect the interactions, their durability under various ionic strengths or different pH, and try to elucidate the mechanism of formation of the HA-amino acid ionic pair, as well as to propose the geometry and most probable 3D form of such product.

2. Experimental

2.1. Materials

Sodium hyaluronate (HA) of technical grade was purchased from Contipro Group s.r.o. and supplied in two molecular weights determined by the company and noted as an interval of 1.75–2 MDa ($\cong 10^6$ g mol^{-1}) for the high molar mass HA, and as 70–90 kDa ($\cong 10^3$ g mol^{-1}) for the low molar mass HA. Lysine, Arginine and 6-Aminocaproic acid in p.a. quality were purchased from Sigma–Aldrich, Steinheim, Germany. NaCl, Na_2HPO_4 and NaH_2PO_4 of analytical grade were obtained from Lach-Ner s.r.o., Czech Republic. For the preparation of the solutions, water for injections (distilled and sterilized) produced by Fresenius Kabi Italia, Verona, Italy was utilized.

2.2. Samples preparation

All the solutions were prepared at the laboratory temperature (≈25 °C). The HA stock solutions of the concentrations of 0.02, 0.1, 0.2, 0.4% and 0.5% w/w were prepared by weighing the appropriate amount of the polymeric powder and its subsequent and step-by-step addition into a certain volume of pure water alternated with a pipetting of another volume of water at moderate stirring. This procedure was repeated until the whole weight of the polymer was consumed up. After that, the rest of the water was added up to the desired volume. Such a mixture was left stirred for a minimum of 24 h. The stock solutions of the amino acids and the salts were prepared by the direct addition of the whole appropriate weight into pure water and stirred properly for ca 15 min. Subsequently, this admixture was quantitatively transferred into a volumetric flask and filled up by pure water to the mark and thoroughly shaken. For the preparation of the buffer, 0.02 M aqueous solutions of both Na_2HPO_4 and NaH_2PO_4 were firstly prepared. Afterwards, the solution of NaH_2PO_4 was slowly added into the solution of Na_2HPO_4 at simultaneous stirring, until the pH 6 was achieved. The final volume ratio NaH_2PO_4:Na_2HPO_4 was approximately 12:88. For the measurements in 0.15 M NaCl and the buffer, HA and the amino acids were directly prepared in these solutions.

The mixtures of HA and the amino acids in water were prepared by pipetting of a necessary volume of the amino acid stock solution into 10 mL of 0.1 or 0.5% w/w HA solution at simultaneous stirring and a volume of pure water in order to achieve the desired concentration of all components. The admixtures were left stirred overnight. For the study of the ionic strength influence, 5 mL of the HA stock solution of double the concentration was used. After the addition of the amino acid solution, 5 mL of NaCl stock solution was added in order to achieve the desired concentrations of all the components in the final samples.

2.3. Rheological measurements

The rheological measurements were carried out using AR-G2 rotational rheometer (TA Instruments) at 25 °C controlled by a Peltier system. Two types of geometries were used according to the sample's viscosity; i.e. high molar mass HA solutions were measured by using cone-plate (CP) geometry of the diameter of 60 mm and the cone angle of 1° and with the solvent trap system against the evaporation of the sample. Low molar mass HA solutions were measured by the double gap concentric cylinders geometry. The solutions were applied to the geometries using a pipette and were conditioned at 25 °C and within the measuring gap for 5 min prior to the measurement. Firstly, the *Continuous ramp* test for a brief investigation of the sample's flow behavior was applied. In this test, the shear rate was continuously raised from 0.1 to 1000 s^{-1} lasting 5 min. Secondly, the *Steady-state* test was applied to the fresh sample with a varied shear stress ranging from 0.01 to 10 Pa with the percentage tolerance of 5%, sample period of 10 s and three consecutive repetitions. This test was applied at least two times on always freshly added sample.

2.4. Electrical conductance

The electrical conductance, *G*, was measured using Mettler Toledo conductometer by gentle immersion of the conductance electrode into the solution. The auto temperature control (ATC) function was turned on and set up to 25 °C. The values were read in 10 s intervals until 5 stable values were obtained, i.e. the values differed by less than 5% from each other and from them the mean value of *G* was calculated. The measurements were performed as